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Behavior of DNA under hydrothermal conditions with $MgCl_2$ additive using an *in situ* UV–vis spectrophotometer

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Abstract

The capillary flow hydrothermal reactor system for the UV–vis spectrophotometric detection system (CHUS) has been applied to inspect the behavior of DNA at temperatures up to 300 °C. Double-stranded DNA (dsDNA) was monitored with and without ethidium bromide in the presence of $MgCl_2$. The ratio (A_{+DNA}/A_{-DNA}) of the absorbance of EB with DNA (A_{+DNA}) to that without DNA (A_{-DNA}) increased steeply over 1 at temperatures around 100 °C, which was not observed in the absence of $MgCl_2$. This was found to be due to the decrease of solubility of DNA in hot water, where dsDNA is converted to single-stranded DNA and become insoluble. At temperatures over 175 °C, DNA becomes soluble again because of the degradation of long insoluble DNA polymers to short soluble DNA oligomers. This study points out the importance of solubility of DNA for life at extremely high temperatures as well as the stability of DNA.

Keywords: *In situ* UV–vis spectrophotometry; Hydrothermal reaction; Stability of DNA; Solubility of DNA; Chemical evolution; Hydrothermal origin of life

1. Introduction

Hydrothermal reactions are becoming important in both fundamental and practical areas. Nowadays, it is realized that hydrothermal environments are indispensable for some special biochemical reactions. First, the hypothesis that life could have been originated under hydrothermal environments (the hydrothermal origin of life hypothesis) has been proposed. The hydrothermal origin of life hypothesis is supported by the phylogenetic analysis of the present living-organisms and the simulation experiments under submarine vent conditions on the earth [1–6]. The last common ancestor (LCA) of the present organisms is considered as a hyperthermophilic organism [1–3]. In addition, geological records suggest that the primitive Earth environments resembled the modern hyperthermophilic biotopes when oldest organisms were present [7–11]. However, the nature of LCA is not yet commonly accepted [12–15]. Furthermore, the hydrothermal origin of life hypothesis seems to be inconsistent with the RNA world hypothesis [16–20]. That is to say, it is considered that nucleotides are labile under redox constrained hydrothermal conditions [12,21–26] although some minerals are capable to protect nucleotides and their precursors from their degradations [27]. Additionally hydrophobic interaction and hydrogen bonding within polynucleotides would not be effective to preserve biological information and catalytic functions at high temperatures [21,28,29]. Although the hydrothermal origin of life hypothesis has been still disputed, these speculations need to be experimentally verified.

Second example of hydrothermal reactions related with biological science can be found in the biochemistry of hyperthermophilic organisms. Presumably, DNA molecules are protected by several mechanisms in modern hyperthermophiles [30], such as specific proteins [31], polyamines [32,33], increase of G–C ratio [34]. However, the melting of naked dsDNA molecules normally proceeds at temperatures below 100 °C [35,36]. Thus, it would be essential to evaluate how tertiary structures and covalent bonding within DNA molecules could be stabilized in thermophiles at high temperatures near and over 100 °C.

Nevertheless, there has been no practical technique to evaluate the behaviors of biomolecules at extremely high temperatures. In addition, there are few studies on the solubility of

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biopolymers under hydrothermal conditions while biopolymers should be protected from their aggregation and precipitation even under hydrothermal conditions for preserving replication and catalytic functions in hyperthermophiles. These drawbacks concerning the origin of life and the biochemistry of thermophilic organisms have been experimentally verified by our new techniques for hydrothermal reactions [20,25,26,28,29,37], such as by the capillary flow hydrothermal reactor system for the UV–vis spectrophotometric detection system (CHUS) to investigate hydrothermal reactions [38]. This technique was applied for evaluation of the behavior of DNA at 25–200 °C using the intercalation of ethidium bromide (EB) to dsDNA [29]. However, it was unexpected that the absorbance of EB at 110–135 °C was increased in the presence of phosphate or borate pH buffer. The scope of this unknown phenomenon has been continuously investigated.

In the present study, the observation of DNA behavior with $MgCl_2$ additive under the hydrothermal environments was attempted using CHUS to evaluate the stability and the tertiary structure of DNA at temperatures up to 300 °C since $MgCl_2$ potentially stabilizes dsDNA [36]. Based on the results, the importance of solubility of DNA molecules for life at extremely high temperatures is discussed.

2. Experimental

2.1. Materials and equipment

DNA from salmon testes and EB were purchased from Wako Pure Chemical Industries Ltd., Tokyo. 2-Amino-2-hydroxymethyl-1,3-propanediol (Tris) was obtained from Kanto Chemical, Tokyo. All other reagents used were of analytical grade. Fused-silica capillary tubing was obtained from GL Science, Tokyo.

2.2. Sample preparation

A sample solution containing 5.0×10^{-3} M DNA, 5.0×10^{-4} M EB, 0.2 M $MgCl_2$ and 0.01 M Tris buffer (pH 7.9) (Sample 1) and that containing 5.0×10^{-4} M EB, 0.2 M $MgCl_2$ and 0.01 M Tris buffer (pH 7.9) (Sample 2) were prepared. Besides, a sample solution containing 5.0×10^{-3} M DNA and 0.01 M Tris buffer (pH 7.9) (Sample 3) and that containing 5.0×10^{-3} M DNA, 0.2 M $MgCl_2$ and 0.01 M Tris buffer (pH 7.9) (Sample 4) were prepared for direct measurements of UV absorption of DNA. In addition, a sample solution containing 5.0×10^{-4} M EB, 0.2 M $MgCl_2$ and 0.01 M Tris buffer (pH 7.9) (Sample 5) and a sample solution containing 2.5×10^{-3} M DNA and 0.01 M Tris buffer (pH 7.9) (Sample 6) were prepared for the direct measurements of absorbance change of DNA at high temperatures. The solutions containing DNA and 0.2 M $MgCl_2$ did not form precipitates at room temperatures.

2.3. Apparatus

The CHUS system was set up as described in the previous study [29,37,38]. The system was composed from a solvent reservoir, a high-pressure pump, a high-temperature reactor, a temperature controller, a UV–vis detector (Model UV-1575, JASCO Corporation, Japan), back-pressure equipment, and a data integrator (Fig. 1). The high-temperature reactor consists of heating blocks, capillary tubing, and a detection interface unit. A fused-silica capillary tubing of 0.1 mm inner diameter and about 600 mm length (GL Science, Tokyo, effective length 340 mm, the effective volume 2.67×10^{-3} cm^{-3}) without any treatment of the inner wall was used as a flow reactor tubing. The flow rate was controlled at 0.125–0.5 mL min^{-1}, so the heating time that the sample solutions were exposed at a high temperature was altered in the range of 0.32–1.28 s. The heating time is sufficiently long compared with the time required for heating up the sample solutions (ca. 40 ms) [37]. The temperature was controlled at 25–300 °C within ±1 °C and the pressure was controlled at over 10 MPa. Sample solutions were injected more than three times in the CHUS system through a 0.1 mL loop injector, and the absorbance was recorded at 481 nm for the measurements using EB.

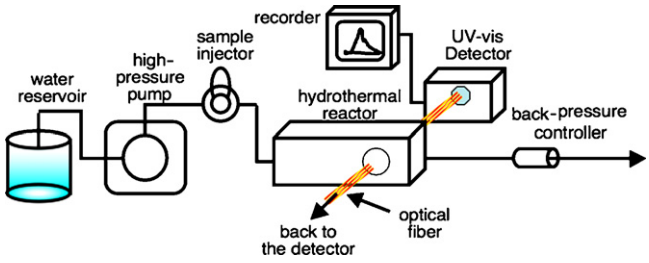

Fig. 1. *In situ* UV–vis spectrophotometric system for hydrothermal reactions.

Besides, the direct measurements of UV absorbance of DNA were attempted at 270 nm. In addition, absorbance changes were measured at 20 nm intervals to obtain absorption spectra at 400–600 nm since the absorbance was too low to obtain absorption spectra directly using the scanning function of the UV–vis detector. In addition, absorption spectra at 25 °C were measured using a Shimadzu UV 240 spectrophotometer with a 0.1 cm quartz cell for the comparison of data obtained by CHUS and the conventional spectrophotometer.

High-performance liquid chromatography (HPLC) was carried out using a LC10A HPLC system (Shimadzu, Japan) with a DNA–NPR anion-exchange column from TOSOH Co., Japan using a gradient of 0.45–1.5 M NaCl at pH 11 with 0.02 M Tris buffer within 10 min at 35 °C.

3. Results and discussion

3.1. Behavior of DNA in aqueous solutions at extremely high temperatures

EB possesses absorption band at 400–600 nm and the absorption at maximum wavelength (481 nm) decreases in the presence of dsDNA and remains constant at dsDNA over 5 mm [29]. The decrease of absorbance at 481 nm with dsDNA reflects the intercalation of EB to dsDNA. No precipitate was detected in the mixtures including DNA in the presence of $MgCl_2$ although DNA potentially forms precipitate or aggregate in the presence

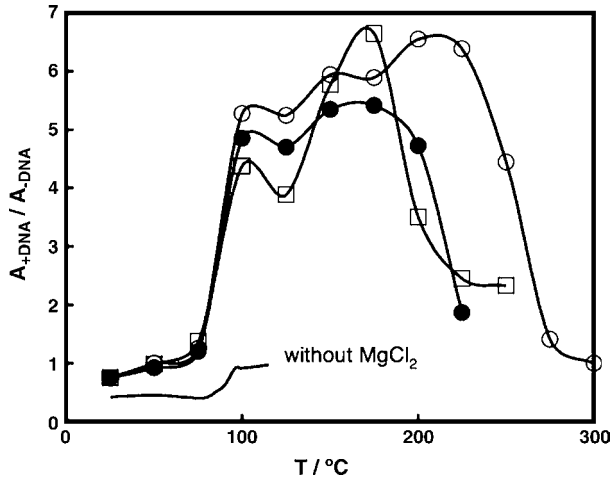

Fig. 2. Relationship between A_{+DNA}/A_{-DNA} and temperature on the basis of indirect measurements using EB. A_{+DNA}/A_{-DNA} = (absorbance in the presence of DNA)/(absorbance in the absence of DNA). Sample 1: 5.0×10^{-3} M DNA, 5.0×10^{-4} M EB, 0.2 M $MgCl_2$ and 0.01 M Tris buffer (pH 7.9); Sample 2: 5.0×10^{-4} M EB, 0.2 M $MgCl_2$ and 0.01 M Tris buffer (pH 7.9). Sample 1 gives absorbance in the presence of DNA and Sample 2 gives absorbance in the absence of DNA. Wavelength: 480 nm. Heating time—open circles: 0.32 s, closed circles: 0.64 s and open squares: 1.28 s. The solid line is obtained in the previous study without $MgCl_2$ [29].

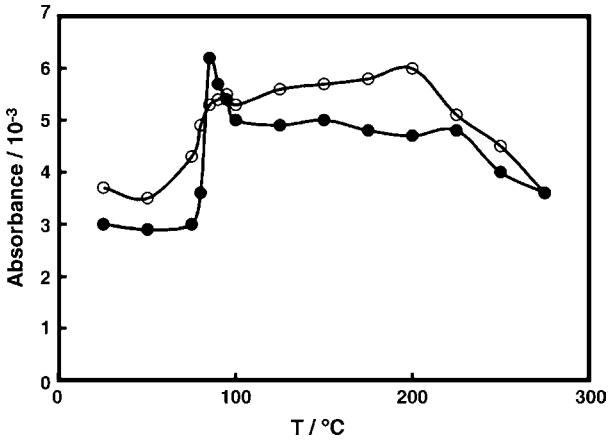

Fig. 3. Absorbance vs. temperature on the basis of direct measurements of UV absorption of DNA. Conditions—open circles (Sample 3): 5.0×10^{-3} M DNA and 0.01 M Tris buffer (pH 7.9); closed circles (Sample 4): 5.0×10^{-3} M DNA, 0.2 M $MgCl_2$ and 0.01 M Tris buffer (pH 7.9). Wavelength: 270 nm. Heating time: 0.64 s.

of Mg^{2+} ions [39–45]. Absorbance (A_{+DNA}) of EB in the presence of DNA and that (A_{-DNA}) in the absence of DNA was monitored at 481 nm at 25–300 °C using the CHUS system. Then, the values of A_{+DNA}/A_{-DNA} were calculated. Relationships between A_{+DNA}/A_{-DNA} versus temperature are shown in Fig. 2. The values of A_{+DNA}/A_{-DNA} slightly increased at 25–75 °C and steeply increased at 75–100 °C. The value of A_{+DNA}/A_{-DNA} roughly remained constant at 100–175 °C and dropped again to 1 at temperatures over 175 °C.

Principally, the magnitude of A_{+DNA}/A_{-DNA} should approach to 1 with increasing temperatures since EB releases from dsDNA. The increase of A_{+DNA}/A_{-DNA} at 75–100 °C reflects that EB is released from DNA since dsDNA is converted to single-stranded DNA (ssDNA). This is consistent with the previous study using different pH buffer solutions without $MgCl_2$; the melting temperature in this case was less affected by the presence of $MgCl_2$. However, it was unexpected that the values of A_{+DNA}/A_{-DNA} sharply increased over 1 at around 100 °C. The increase of A_{+DNA}/A_{-DNA} in the present study is obviously strong as comparing to the previous case without $MgCl_2$ (solid line in Fig. 2). The values of A_{+DNA}/A_{-DNA} increased up to 4–7 and dropped at 175–275 °C in the present case, whereas those increased up to 1–1.5 and dropped at 125 °C. On the other hand, the fact that A_{+DNA}/A_{-DNA} drops at 175–275 °C in the present case indicates that the interaction between DNA and EB disappears at 175–275 °C. In addition, this drop was dependent on the flow rate of CHUS, whereas other profiles of A_{+DNA}/A_{-DNA} versus temperature at 25–175 °C are basically independent on the flow rate. This is indicative for understanding the phenomenon that the values of A_{+DNA}/A_{-DNA} increased over 1.

By the way, a direct measurement of conformational change of dsDNA to ssDNA at 270 nm was attempted for the first time using CHUS while there are following disadvantages in the CHUS system. First, the sensitivity of CHUS is very low because of its short light path length (0.1 mm) using the fused-silica capillary (Fig. 3). In the present case, fortunately the concentration of DNA was fairly high. Second, the transparency of the optical fibers of CHUS gradually drops at below 280 nm. Thus, the wavelength 270 nm was chosen since DNA bases possess absorption band at 250–280 nm. The direct measurements of absorbance at 270 nm were barely possible in the present CHUS system while 260 nm is normally a better wavelength to detect DNA. The increase of absorbance was observed at 75 °C and remained constant at 100–200 °C, and then slightly decreased at 200–275 °C. This relationship is consistent with the relationship that was indirectly measured using EB. It is noted that both indirect and direct observations would be valuable to analyze the behavior of DNA at extremely high temperatures.

On the other hand, monitoring the absorption spectra of mixtures of DNA and EB was attempted for obtaining detail information on the phenomenon that A_{+DNA}/A_{-DNA} increases over 1. Since the sensitivity of CHUS is not sufficiently high for the measurements of UV–vis spectra, direct measurements of absorption spectra using the scanning function of the detector were not applicable. Thus, absorption spectra of EB with DNA at different temperatures were obtained by measuring the absorbance with 20 nm intervals between 400 and 600 nm as shown in Fig. 4. The absorption spectra at 25–50 °C showed the identical spectra to that obtained at 25 °C using a conventional spectrophotometer, where the occurrence of intercalation of EB into dsDNA was observed. The absorption spectrum at 75 °C indicates that the intercalation between DNA and EB is disappearing. On the other hand, the absorption spectra at 100–200 °C showed strong but broad background at 400–600 nm, where the broad background overlies the absorption of EB. In addition, the background absorption involves a large fluctuation for each measurement. Obviously, this type broad and fluctuating absorption is due to the background scattering by the turbidity of sample solutions. However, DNA

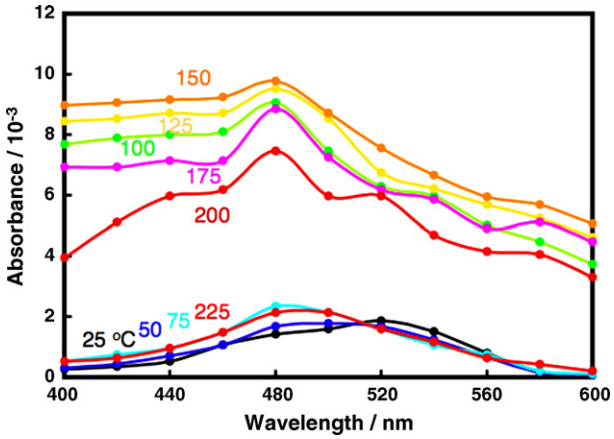

Fig. 4. Absorption spectra vs. temperature for a solution containing EB and DNA. Conditions: 5.0×10^{-3} M DNA, 5.0×10^{-4} M EB, 0.2 M $MgCl_2$ and 0.01 M Tris buffer (pH 7.9). Heating time: 0.64 s.

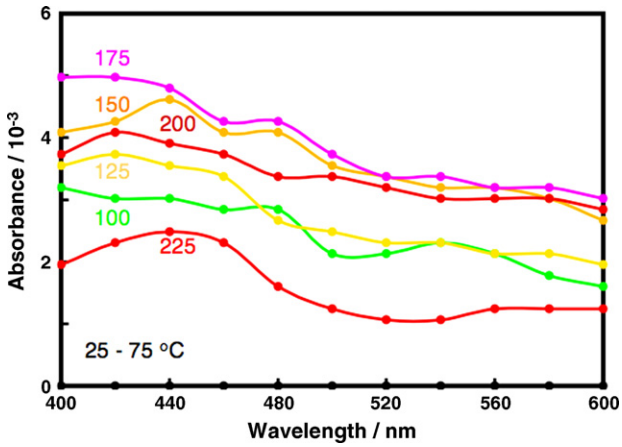

Fig. 6. Absorption spectra vs. temperature for a solution containing DNA. Conditions: 5.0×10^{-3} M DNA, 0.2 M $MgCl_2$ and 0.01 M Tris buffer (pH 7.9). Heating time: 0.64 s.

should be well suspended since the flow in CHUS has never stuck.

To evaluate the turbidity of sample solutions, the absorption spectra of control sample solutions containing either EB or DNA alone were measured (Figs. 5 and 6). First, the absorption spectra without DNA, where the heating time was 0.64 s, showed that there is no background turbidity. The absorption spectra of EB are less influenced at 25–150 °C and slightly shifted to longer wavelength at 200 °C. This fact suggests that EB is almost stable even for 0.64 s at 200 °C. In addition, the absorption spectra can be accurately determined in the absence of turbidity. Furthermore, this fact indicates the molar absorptivity of each species is less influenced at these temperatures. This is indeed true for the case of EB in the presence of DNA, where the absorption spectra of EB at 75 and 225 °C are almost identical as shown in Fig. 4.

Second, the absorption spectra of DNA without EB showed very broad absorption background at 400–600 nm. The origin of these backgrounds is regarded as same as that obtained for the mixture of DNA and EB. Naturally, the background scattering by turbidity involves large fluctuations so that the background for each measurement cannot be identical. Conclusively, the broad absorption at 400–600 nm in the EB and DNA mixture at temperatures over 100 °C is due to the presence of DNA, where DNA resulted in turbidity. The turbidity is probably due to the decrease of solubility of DNA at high temperatures. That is to say, dsDNA is converted to ssDNA at 75–100 °C and then DNA and Mg^{2+} ions would form insoluble precipitate or aggregate [39–45]. The solubility of ssDNA is lower than that of dsDNA especially in the presence of Mg^{2+}. Moreover, while the A_{+DNA}/A_{-DNA} values remain roughly constant at 100–175 °C at the heating time 0.64 s, the A_{+DNA}/A_{-DNA} values increased at the heating time 0.32 s. This might indicate that the solubility of ssDNA decreases with increasing heating time. Although measurements of absorption spectra in the presence of the turbidity by insoluble ssDNA showed difficulties (Figs. 4 and 6), measurements without turbidity were accurately performed as shown in the cases at low temperatures in Fig. 2 and that for the spectra of EB in Fig. 5. In particular, the absorption spectra of EB were clearly determined even at 200 °C. In addition, the absorption spectra of the mixture of EB and DNA at 225 °C is identical to that measured at 75 °C. Our previous study showed that the absorption spectra of a water-soluble porphyrin possessing very high molar absorptivity (5.0×10^5 cm^{-1} M^{-1}) can be determined accurately. By the present study, it was also confirmed that the accurate measurement of UV–vis absorption spectra at high temperatures is also possible for substances possessing a much lower molar absorptivity of which EB was 5.7×10^3 cm^{-1} M^{-1} at 481 nm in the presence of $MgCl_2$.

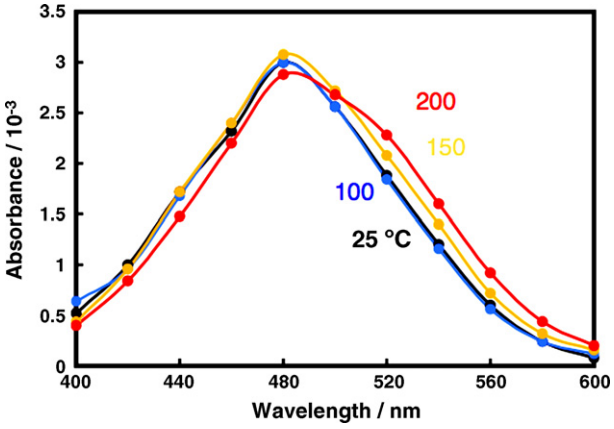

Fig. 5. Absorption spectra vs. temperature for a solution containing EB. Conditions: 5.0×10^{-4} M EB, 0.2 M $MgCl_2$ and 0.01 M Tris buffer (pH 7.9). Heating time: 0.64 s.

The fact that the A_{+DNA}/A_{-DNA} dropped at over 175 °C can be understood as follows on the basis of the presence of insoluble ssDNA at high temperatures. The starting temperature of the decrease of A_{+DNA}/A_{-DNA} at 175–275 °C is reduced with increasing the heating time of solutions exposed at high temperatures (Fig. 2). This fact indicates that ssDNA becomes soluble with increasing the heating time at high temperatures. That is to say, long ssDNA molecules were degraded to short ssDNA molecules including DNA monomers. The short ssDNA including monomers possesses higher solubility than the starting DNA

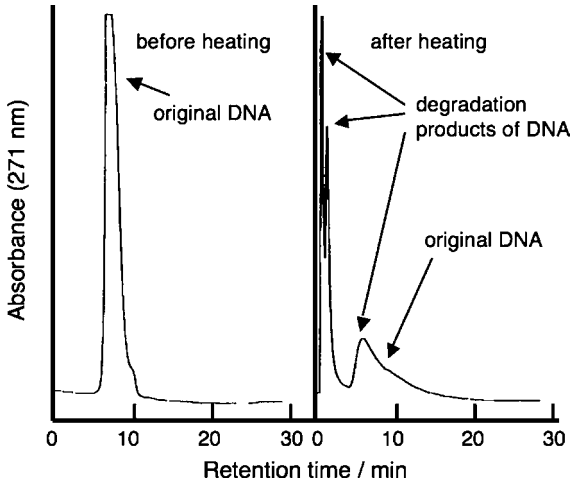

Fig. 7. Anion-exchange HPLC profiles for the solution of DNA. Samples: 5.0×10^{-3} M DNA, 0.2 M $MgCl_2$ and 0.01 M Tris buffer (pH 7.9). Left: before heating, right: after heating for 0.64 s at 275 °C. HPLC conditions: a gradient of 0.45–1.5 M NaCl within 10 min with 0.02 M Tris buffer at pH 11 at 35 °C, detection: 270 nm.

polymers. This is dependent on the decomposition rate of ssDNA so that the values of A_{+DNA}/A_{-DNA} decreased with increasing the heating time.

To obtain a further evidence of degradation of DNA mentioned above, the samples exposed for 0.64 s at 275 °C were analyzed by anion-exchange HPLC, where the retention time of oligonucleotides increases with increasing the length of oligonucleotides. It was confirmed that DNA was degraded to shorter oligonucleotides (Fig. 7), where a very small amount of original DNA is observed as a shoulder peak near the partially degraded DNA. In addition, the turbidity of a sample solution containing DNA was observed in a glass vial when the sample was taken out as soon as possible after the sample was heated up for a few minutes at 120 °C in a cartridge heater.

Conclusively, dsDNA melts to ssDNA at 75–100 °C and ssDNA becomes insoluble under hydrothermal conditions. In particular, the solubility of ssDNA can be reduced in the presence of Mg^{2+} on the basis of the comparison between the present and previous studies [26]. The turbidity by insoluble DNA in aqueous solutions at high temperatures was visualized as absorption spectral changes for the first time using the CHUS system.

3.2. Implications on the behavior of DNA in life-like systems at high temperatures

This study demonstrated that the stability and solubility of naked DNA seem to be insufficient for preserving information at high temperatures over 100 °C, where the secondary structure of dsDNA would be readily destroyed by degradation and/or aggregation. The cleavage of phosphodiester bonding within ssDNA starts at 175–225 °C when the heating time is 0.32–1.28 s. Besides, the present study demonstrated the importance of the solubility of DNA for life-like systems, such as in modern hyperthermophiles and possible origin of life systems under hydrothermal conditions. The solubility of DNA steeply decreased at higher temperatures beyond the melting point since dsDNA is converged to ssDNA, which possess low solubility in aqueous medium at high temperatures. This phenomenon was also enhanced with $MgCl_2$. This fact indicates that bare DNA molecules could not be readily dissolved in physiological media at high temperatures. Thus, it is important to investigate whether the aggregation of DNA could have resulted in disadvantageous for the biological functions of DNA molecules. Similarly, the solubility of biomolecules, such as RNA molecules and proteins, under hydrothermal conditions would be also important for the emergence of biological functions.

These facts support that the survival and the occurrence of biological functions of bare DNA molecules are difficult under the hydrothermal vent conditions from both viewpoints concerning the biochemistry of hyperthermophiles and the hydrothermal origin of life hypothesis. Besides, the degradation and secondary structures of DNA are protected in hyperthermophiles by several mechanisms [30]. Thus, entirely naked DNA molecules are not capable to preserve biological functions under hydrothermal conditions unless such machinery is present to stabilize the secondary structures and enhance the strength of covalent bonding of DNA molecules.

4. Conclusions

The absorbance change and absorption spectra of solutions containing DNA with and without EB were successfully monitored for the first time in the presence of $MgCl_2$ additive at 25–300 °C using CHUS. An unexpected phenomenon that the absorbance of intercalator EB in the presence of DNA notably increased at over 100 °C was evaluated by the measurements of UV–vis absorbance and absorption spectra. It was deduce that the phenomenon is due to the conversion of dsDNA to ssDNA, the less solubility of ssDNA, and the degradation of ssDNA at 100–300 °C. The present investigations demonstrated for the first time that DNA molecules have disadvantages from both the viewpoints of the stability and solubility as essential biopolymers for life at extremely high temperatures. In other words, this paper elucidated that the CHUS system is powerful tool for monitoring the solubility of biomolecules at extremely high temperatures since the solubility of such biomolecules can be hardly known by conventional techniques.

Acknowledgements

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